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Telomerase and Chromosome End Protection In Vivo: the TPP1 Connection

Eric Gilson[1,2,3,*] and M. Teresa Teixeira[1]
[1]Laboratoire de Biologie Moléculaire de la Cellule, Université de Lyon, CNRS UMR5239, Ecole Normale Supérieure de Lyon, 46 allée d'Italie, F-69364 Lyon Cedex 07, France
[2]Laboratory of Biology and Pathology of Genomes, University of Nice, UMR 6267, CNRS U998 INSERM, 28 Avenue Valombrose, Faculté de Médecine, 06107 Nice Cedex 2, France
[3]Department of Medical Genetics, Archet 2 Hospital, CHU of Nice, France
*Correspondence: Eric.Gilson@ens-lyon.fr
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In an article published in this issue of *Developmental Cell*, Maria Blasco's group shows that the telomere end-binding protein TPP1 is involved in both end protection and telomerase regulation in vivo. Importantly, they highlight the relevance of telomerase activity in highly proliferative tissues and in reprogramming of cells to induced pluripotency (iPS).

The linear chromosomes of eukaryotes end with telomeres that are essential for chromosome stability. Telomeres are composed of short DNA repeats that protrude to form a single-stranded 3′ tail, a set of proteins that specifically binds to them, and telomeric transcripts. All these elements are required to ensure telomere protection from inappropriate fusion or degradation. Because of the inability of semiconservative DNA replication machinery to fully duplicate DNA ends, telomeres display a singular dynamic structure, providing also a mode of control of cell division in eukaryotes: as telomeres erode, cells eventually loose viability, leading to senescence. This has been considered a major pathway for aging in humans, and also a potent barrier for tumor progression.

In proliferating cells, such as germ and stem cells, telomere erosion is compensated mainly by elongation by the expression of a specialized reverse transcriptase, telomerase. This enzyme is a ribonucleoprotein that also contains a noncoding RNA, so-called telomerase RNA, that contains a small region used as a template to iteratively synthesize telomeric repeats.

In the current model for telomerase action, best described in *S. cerevisiae* at the end of S phase, short telomeres activate a DNA damage-like pathway (Sabourin and Zakian, 2008). Telomerase is recruited to chromosome termini via its Est1 subunit, which binds directly to Cdc13, a protein associated with the telomeric 3′ single-stranded tail; a chimeric protein fusing the catalytic moiety of telomerase to Cdc13 can bypass the requirement for Est1 (Evans and Lundblad, 1999). Interestingly, Cdc13 also forms a complex with Stn1 and Ten1, analogous to the single-stranded DNA-binding "replication protein A" (RPA) complex involved in DNA replication and repair. In this context, Cdc13 is essential to prevent the degradation of the 5′ end-containing strand of telomeres. Curiously, this dual role in both telomere protection and telomerase recruitment is shared with another single-stranded binding protein complex of telomeres, POT1-TPP1.

In fact, in ciliates and humans, a different family of single-stranded binding proteins is involved in telomere protection and in the recognition of telomeres by telomerase. The complex POT1-TPP1 (homologous to the ciliate TEBP alpha and beta, respectively) also contains the predicted structural protein domains (OB-folds) present in Est1, Cdc13, Stn1, Ten1, and RPA subunits. As in yeast, OB-fold proteins of other eukaryotes (e.g., POT1-TPP1) have been implicated in the connection between telomerase and telomeres (Wang et al., 2007; Xin et al., 2007). First, TPP1 enhanced the association of POT1 and single-stranded telomeric DNA. Second, the interaction between POT1 and TPP1 was required for telomere lengthening in live cells. Finally, telomerase processivity is associated with the POT1-TPP1 complex. More recently, experiments in vitro in Tom Cech's laboratory, using DNA primers as substrate, further refined the biochemical parameters of POT1-TPP1 function in telomerase activity. The POT1-TPP1 complex reduced the primer dissociation rate from telomerase and promoted telomerase translocation (wherein telomerase RNA realigns relative to the product of the reaction to allow iterative telomeric repeat synthesis). Importantly, POT1-TPP1 complex increased telomerase processivity, even if provided substoichiometrically relative to the substrate. This means that if telomerase is limiting, it acts processively preferentially on primers bound to POT1-TPP1, further supporting a model of recruitment of telomerase (Latrick and Cech, 2010).

In this issue of *Developmental Cell*, a paper from Maria Blasco's team examines the in vivo functions of TPP1 (Tejera et al., 2010). The authors show that in addition to a role in protection of telomeres, TPP1 is required for telomerase-dependent telomere lengthening in live mice. In the absence of TPP1, chromatin immunoprecipitation experiments revealed a loss of telomerase localization at telomeres. Telomere lengthening was also impaired, especially during the reprogramming of iPS cells, known to depend on telomerase activity. In vivo, *tpp1*-negative mice showed signs of advanced senescence that were rescued by the inhibition of p53, a known effector of senescence mediated by telomere erosion. The role of TPP1 in telomerase recruitment has also been shown recently in human cells (Abreu et al., 2010). However, the bridge to telomeres seems to depend on other shelterin components,

namely TIN2, rather than on POT1. Altogether, these results converge to a model where TPP1 in vivo, similarly to the in vitro findings, binds to telomeres and connects them to telomerase, and this activity may evolve different shelterin subcomplex rearrangements.

Other proteins binding to the single-stranded region of telomeres also may be involved in modulation of telomerase activity. For instance, an additional OB-fold-containing protein, related to an RPA large subunit, was found to be associated with a highly processive form of telomerase in the ciliate *Tetrahymena thermophila* (Min and Collins, 2009). Similarly, a novel protein factor, CTC1, was described recently at telomeres (Miyake et al., 2009; Surovtseva et al., 2009). This single-stranded binding protein found in plants and humans shares sequence similarities with Cdc13 and possesses functions in telomere protection. However, it remains to be examined whether it is also involved in telomerase-dependent telomere length homeostasis.

The question to answer now is how TPP1 coordinates both end protection and telomerase recruitment, and how this duality is eventually regulated as a function of telomere length in normal somatic cells. Likewise, how is this balance eventually altered in cancer cells? Indeed, if TPP1-POT1 is required for protection, are all telomeres bound to it? Do TPP1-containing complexes invariably serve both roles at all bound telomeres? Future experiments in the field will certainly shed new lights on this aspect of the increasingly complex and dynamic world of chromosome ends.

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A Deathly DNase Activity for Dicer

Katsutomo Okamura[1] and Eric C. Lai[1,*]
[1]Sloan-Kettering Institute, Department of Developmental Biology, 1275 York Ave, Box 252, New York, NY 10065, USA
*Correspondence: laie@mskcc.org
DOI 10.1016/j.devcel.2010.05.004

Recently reporting in *Science*, Nakagawa et al. describe an unexpected role for Dicer in chromosome fragmentation during apoptosis in *C. elegans*. They find that cleavage of DCR-1 by the caspase CED-3 redirects its regulatory activity, by destroying its dsRNase activity while activating an intrinsic DNase activity.

RNase III enzymes are a widely distributed family of double stranded RNA (dsRNA)-specific ribonucleases. Since the discovery of *E. coli* RNase III in the 1960s, the functions of this protein family in ribosomal RNA biogenesis and mRNA decay or regulation have been well studied in bacteria and yeast (MacRae and Doudna, 2007). Importance of their homologs in higher eukaryotes was recognized only in the last decade. In particular, Dicer-family RNase III enzymes are central to the biogenesis of Argonaute-associated small regulatory RNAs, including microRNAs (miRNAs) and small interfering RNAs (siRNAs). Because miRNAs play important roles in diverse biological settings, Dicer genes are essential for many aspects of development and physiology. The cell death pathway has critical connections with the miRNA pathway, since many individual miRNAs have proapoptotic or anti-apoptotic activities. Deregulation of such miRNAs may contribute to various human cancers (Garzon et al., 2009).

Apoptosis is accompanied by DNA fragmentation, which can be visualized by TUNEL (terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling) staining. In mammals, the endonuclease DFF40 (also known as CAD) initiates DNA fragmentation (Widlak and Garrard, 2005). The DNase activity of DFF40 is normally inhibited by DFF45 (also known as ICAD), but when the cysteine protease caspase-3 is activated, it cleaves DFF45 to release active DFF40. Despite strong conservation of the cell death pathway in *C. elegans*, including the functional caspase-3 ortholog CED-3 (Miura et al., 1993; Yuan et al., 1993), its genome does not appear to encode homologs of DFF40 and DFF45. Nevertheless, as apoptotic cells in *C. elegans* exhibit DNA fragmentation (Parrish and Xue, 2006), some nuclease activity is apparently responsible for initiating this process in nematodes.